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Patent claims

1. A DNA sequence coding for hG-CSF characterized in that the sequence comprises the nucleotide sequence of SEQ ID NO: 1.
2. A DNA sequence characterized in that the sequence comprises a nucleotide sequence selected from the group comprising:

   (a) a partial sequence of SEQ ID NO: 1;

   (b) nucleic acids which hybridize with the sequence of SEQ ID NO: 1 under stringent conditions; and

   (c) at least one and preferably a combination of the following modifications with respect to the native hG-CSF sequence:

   - in a "segment I" (located at the 5' terminal end between the nucleotide positions 3 and 194): a plurality of replacements which include replacements of *E. coli* rare codons by *E. coli* preference codons and replacements of GC rich regions by AT rich regions,
   - in a "segment II" (located between the nucleotide positions 194 and 309): a plurality of replacements of *E. coli* rare codons by *E. coli* preference codons,
   - in a "segment III" (located between the nucleotide positions 309 and 467): no change or essentially no change,
   - in a "segment IV" (located at the 3' terminal end between the nucleotide positions 467 and 536): a plurality of replacements of *E. coli* rare codons by *E. coli* preference codons.
3. The DNA sequence according to claim 2, which encodes for a biologically active G-CSF.
4. The DNA sequence according to any one of claims 1 to 3, wherein the nucleotide sequence is capable of providing an expression level of at least 50%, preferably at least 52% in an expression system.
5. The DNA sequence according to claim 1 or 2, further comprising the 5'-untranslated region of the hG-CSF gene which are not changed relative to the native hG-CSF gene.
6. An expression plasmid, characterized in that the plasmid comprises the DNA sequence according to claim 1 or 5 and a plasmid vector.

7. An expression plasmid, characterized in that the plasmid comprises a DNA sequence according to any one of claims 2 to 5 and a plasmid vector.
8. The expression plasmid according to claim 6 or 7, characterized in that the plasmid vector comprises a T7 promoter sequence.
9. The expression plasmid according to claim 6 or 7, characterized in that the plasmid vector is selected from the group of pET vectors.
10. The expression plasmid according to any one of claims 6 to 9, characterized in that the plasmid vector comprises a resistance gene, preferably an ampicilline or a kanamycine resistance gene.
11. An expression system for the expression of DNA sequence according to claim 1, characterized in that the system comprises the expression plasmid according to any one of claims 6 and 8 to 10 and a production strain *E. coli*.
12. An expression system for the expression of the DNA sequence according to claim 2, characterized in that the system comprises the expression plasmid according to any one of claims 7 and 8 to 10 and a production strain *E. coli*.
13. The expression system according to claim 11 or 12, characterized in that the production strain is *E. coli* BL21 (DE3).
14. The expression system according to any one of claims 11 to 13, characterized in that it is used without an antibiotic.
15. A process for construction of DNA sequence according to claim 1 or claim 2, characterized in that the process comprises
    (i) applying methods in order to provide a DNA sequence which is changed relative to the native sequence coding for hG-CSF by:
    -replacement of some *E. coli* rare codons with *E. coli* preference codons, and/or
    -replacement of some GC rich regions with AT rich regions; and
    (ii) maintaining a completely unchanged part in a substantial portion of the native sequence coding for hG-CSF.
16. A process for construction of DNA sequence according to claim 15, wherein the DNA sequence further comprises 5'-untranslated region of the hG-CSF gene, characterized in that the process does not involve changes in the 5'-untranslated region in one or more of the following partial regions: translation initiation region,

ribosome binding site and the region between the start codon and the ribosome binding site.

17. The process for construction of DNA sequence according to claim 15 or 16, wherein a completely unchanged sequence according to (ii) is maintained in segment III in a sequence of at least 99 nucleotides in length.

18. The process for construction of DNA sequence according to any one of claims 15 to 17, further comprising inserting said constructed DNA sequence into a plasmid vector which comprises a T7 promoter sequence.

19. The process for construction of DNA sequence according to any one of claims 15 to18, which constructed DNA sequence is capable of providing an expression level of at least 50%, preferably at least 52% in a suitable expression system.

20. A process for the expression of hG-CSF, comprising expressing the DNA sequence according to any one of claims 1 to 5, or the expression plasmid according to any one of claims 6 to 10 in *E. coli*.

21. The process for the expression of hG-CSF according to claim 20, wherein IPTG is used for induction at a concentration in the range of at least 0.1 mM to less than 1 mM, preferably at a concentration of about 0.3 to 0.6 mM.

22. The process according to claim 20 or 21, which comprises a fermentation step that is performed at a temperature of about 20°C to 30°C, preferably at around 25°C.

23. The process according to claim 20 or 21, wherein the expression level is at least 50%, preferably at least 52%.

24. A process for the manufacture of a pharmaceutical composition containing, as an effective ingredient, hG-CSF or biologically active G-CSF, comprising the steps of:

(a) carrying out a process according to any one of claims 20 to 23,

(b) isolating and/or purifying the hG-CSF or biologically active G-CSF obtained by step (a), and

(c) mixing the isolated and/or purified hG-CSF or biologically active G-CSF with a pharmaceutically acceptable carrier or auxiliary substances.